Vol. XXIII. *October, 1912.* *No. 5*

BIOLOGICAL BULLETIN

THE UTERINE SPINDLE OF THE POLYCLAD PLANOCERA INQUILINA.

J. T. PATTERSON AND H. L. WIEMAN.

I. Introduction.

In the uterine eggs of several species of turbellarians there appears a large conspicuous spindle to which the name "uterine spindle" is applied. It is also known under the terms "disappearing spindle" and "aborting spindle." The appearance of this spindle in the uterine eggs of these worms would not in itself be so striking were it not for the remarkable statements that have been made concerning its subsequent history. It is the general opinion of those who have observed the spindle that upon reaching the metaphase it breaks down to form a resting nucleus, which in turn gives rise to the first maturation spindle.

The uterine spindle was first described in 1881 by Selenka, in connection with his work on *Thysanozoön Diesingii.* He states that it first makes its appearance in the eggs after they have become full grown and have passed into the uterus, and is preceded by changes that are typical of the first maturation mitosis. Thus the chromatin of the germinal vesicle forms a spireme, the achromatic spindle with centrosomes appears, and the chromosomes pass into the equatorial-plate position. At this point the division process is arrested, and the "polar-suns" draw closer together, become indistinct, and the chromosomes fuse. The whole nucleus finally returns to a resting condition. Later, the egg gives off two polar bodies, is fertilized and proceeds to develop in the normal manner. Selenka concludes that this incomplete karyokinesis occurs in order to effect a massing of the yolk granules about the astral centers.

Lang ('84) seems to have been the next observer to have noted

the uterine spindle. He describes it as appearing in several species of polyclads; and while he does not accept Selenka's theory as to its function, nevertheless he regards it as a part of the normal process of the nucleus.

In 1894 Wheeler observed the uterine spindle in the eggs of *Planocera inquilina*, a polyclad inhabiting the branchial chamber of *Sycotypus canaliculatus*. Wheeler was concerned with a description of this new polyclad, and did not attempt to work out the details of the karyokinetic process. Since *Planocera* is the form with which this paper is concerned, we may quote Wheeler's entire but brief statement on the aborting spindle. His statement is as follows: "As soon as the mature ova pass into the uteri a curious phenomenon, first seen by Selenka in the uterine eggs of *Thysanozoön Diesingii*, may be observed. The wall of the germinal vesicle fades away and a spindle is formed with distinct polar suns containing centrosomes. The small chromosomes, nine or ten in number, form an equatorial plate and appear to undergo fission, but of this I am not certain. Then the polar asters grow faint and vanish and the nucleus returns to the resting stage during or just before oviposition. Before the nucleus has returned to the resting stage the spermatozoön enters the egg. I have several times seen the deeply staining and somewhat twisted head of the spermatozoön lying in the cytoplasm near the arrested spindle. Further than this I have not traced the phenomena of impregnation, as my attention was first attracted to them while studying hardened material when I was far from the sea-shore. Why a spindle should be formed in the mature ovum and no division result, but only a return of the nucleus to its resting stage, is not easily understood. The spindle lies in the center of the egg and has nothing to do with the formation of the polar bodies; for these do not appear till some time after the eggs are laid, as I have several times had occasion to observe." Wheeler concludes his account of the spindle by objecting to Selenka's view concerning its supposed function.

Gardiner, '95 and '98, studied the uterine spindle in the acœlan *Polychœrus caudatus;* but in this form it is clearly the first cleavage spindle, as the polar bodies are thrown off before

it makes its appearance. However, according to the description of Gardiner the behavior of this spindle is similar to that of the uterine spindle of the polyclad egg. Thus he states that if the animal be kept too long under adverse conditions, the polar suns of the spindle grow dimmer, draw closer together, and the nucleus appears to return completely to a resting stage. The egg remains in this condition until after it is laid, when the spindle again appears, this time to initiate the process of cleavage.

Through certain experiments, Gardiner, '98, demonstrated that the retrograde growth of the amphiaster of the uterine egg was due to placing the animals under adverse conditions, which in turn caused a delay in the laying of the egg, and under such circumstances development begins, as indicated by the appearance of this spindle. Furthermore, he clearly showed that the uterine spindle of *Polychœrus* follows maturation, and is therefore, as stated above, the first cleavage spindle. Through the results of these experiments, Gardiner is led to suggest that the so-called uterine spindle of the polyclads is probably the first segmentation spindle. His exact position on this point may be gleaned from the following quotation: "I would suggest, therefore, that individuals of the polyclads, in which such structures are found, have before death been placed under some abnormal conditions; that the ovum has been fertilized and the polar bodies formed; that the first segmentation spindle has been formed; and that the environment was such that oviposition could not take place; consequently, that a retrograde development of this spindle has taken place exactly as in *Polychœrus*."

That the ground for this suggestion is not well taken, we shall later try to demonstrate, at least in the case of *Planocera*. The suggestion of Gardiner, however, should make us cautious about regarding as aborting spindles those that are visible in the uterine eggs of several worms figured by von Graff ('82 and '08) and which a number of investigators have cited as examples of the disappearing spindle.

In 1907, Surface, who studied the early development of *Planocera*, also called attention to the uterine spindle in this animal. He did not attempt to work out the history of the spindle, but gives merely an outline figure of a freshly laid egg,

in which is seen what he takes to be a "germinal vesicle" produced by the retrograde development of the uterine spindle. Surface states that if the spindle is an abnormal display, as claimed by Gardiner, it at any rate does not interfere with the normal process of development.

The last account of the uterine spindle is that on the rhabdocœle *Graffilla Gemellipara,* which was studied by one of the present writers (Patterson, '12). In this animal a large conspicuous spindle was occasionally met with in eggs that had not yet undergone maturation. The spindle in *Graffilla* differs from that so far observed in any other worm, for in practically every case it exhibited some peculiar condition, such as the abnormal position of the chromosomes on the spindle fibers, or even their complete absence from the spindle. It was pointed out that on account of the viviparous mode of reproduction, *Graffilla* was not a favorable form in which to study the history of the aborting spindle. It is impossible to secure a complete series which would show conclusively the exact progress of its development.

To sum up: The term uterine spindle has been applied to a variety of karyokinetic phenomena which occur during the first steps of development. Some of these cases are undoubtedly due to abnormal development; others are not, especially those of the polyclads. Here the general verdict seems to be that the uterine spindle appears before maturation, that it does not go beyond the equatorial-plate stage, and that it subsequently retrogrades to produce a sort of resting nucleus, which in turn develops the first maturation spindle.

It is evident from the above brief review of the literature that a great deal of obscurity exists regarding the uterine spindle. In view of the fact that no one has given a consistant account of its development, and in view of the further fact that no rational function has been assigned to it, we have considered it worth while to make a detailed study of this spindle. To do this we have selected *Planocera,* not only because of the ease with which this animal can be secured, but also for the reason that its egg is supposed to contain the most typical example of this apparent anomaly of cytology.

Material and Methods.—Two or three is the most common

number of worms found in a single whelk, although we have secured as many as eight from one specimen. After removing the shell, the branchial chamber of *Sycotypus* is slit open and the worms removed to dishes of fresh sea water. This operation was done very shortly after the animals were brought into the laboratory.

When the eggs are fully matured the polyclad lays within several hours after being transferred; otherwise twelve or even twenty-four hours may elapse before eggs are deposited. Usually the eggs are laid in a helicoid spiral on the bottom or sides of the dish, as Wheeler ('94) and Surface ('07) have observed; but quite often oviposition occurs beneath the surface film of the water, in which case the egg string takes the form of a slightly curved ribbon. In the latter instance the worm lies with its ventral surface upward, in which position one can readily study the entire process under the binocular. The eggs are forced out by rhythmic contractions of the egg ducts, and at the same time embedded in a perfectly transparent gelatinous substance of a very sticky consistency. The average time for the act of oviposition is about 15 minutes. Each egg is provided with a delicate capsule, probably secreted by the shell gland surrounding the egg duct. Occasionally a single capsule incloses two eggs. A string may contain as many as 2,000 eggs. Adult worms were obtained showing eggs in every stage of development from the beginning of the growth period up to the time of laying. Eggs were killed just at time of oviposition and at fifteen minute intervals for several hours afterward. In this way a complete series of stages covering the entire period of growth and maturation was obtained.

Adults and eggs were killed in the bi-chloride-acetic-formalin mixture described by Bartelmez ('12).

SOLUTION 1.

Saturated solution of .7 per cent. NaCl	94 c.c.
Glacial acetic acid	6 c.c.

SOLUTION 2.

Neutral formalin (commercial formaldehyde neutralized with $MgCO_3$)	10 c.c.

The two solutions were kept separate until the time of using.

The worms were killed in the mixture heated to 50° C. and left for one and one half hours. The eggs were treated with the cold solution for about an hour. Excellent fixation was obtained, and the material proved very favorable for cytological study.

When the eggs were laid on the glass, they were allowed to remain until 80 per cent. alcohol was reached, and then carefully taken off with a sharp scalpel. An entire string may be removed in this way without losing or injuring a single egg.

The adults and eggs were embedded and cut in hard paraffin (60° C.) to which sufficient quantity of a rubber-asphaltum-paraffin mixture was added to produce a light amber shade. Sections of 5, 7 and 10 micra thickness were cut without difficulty.

Heidenhain's iron-alum-hæmatoxylin with orange G gave very good results, but the method was in all cases checked by staining parts of series with safranin and Lichtgrün.

II. Formation of the Spindle.

The nucleus of the egg has a very characteristic appearance throughout the growth period. A coarse reticulum containing varying amounts of chromatic material at its nodal points, depending upon the degree of maturity of the ovum, and a large spherical basic-staining nucleolus are always present (Fig. 1).

Fig. 2 shows an early stage in the prophase of the uterine spindle, in which the chromosomes are forming at various points in the reticulum. Our preparations clearly show that the entrance of the spermatozoön into the egg is the stimulus which initiates the process, and that uterine eggs in which a careful examination fails to reveal the presence of a sperm invariably have the nucleus in the resting condition. Owing to the hypodermic method of insemination (Wheeler, '94) all the tissues of the body at this time are filled with spermatozoa which finally work their way to the uterus where they penetrate the ova. In the impregnated ovum the head of the spermatozoön stands out very distinctly as a deeply stained sickle-shaped rod, sharply pointed at one end, so that its presence can be easily recognized.

In Fig. 3 the chromosomes are fully formed in a group about the nucleolus which at this time stains very faintly. The nuclear membrane, though still intact, is somewhat wrinkled

in outline suggesting that certain substances are passing out of the nucleus into the cytoplasm.

Fig. 4 shows the chromosomes scattered irregularly on the spindle, and it will be noted that the chromosomes are of a bivalent type. The centrosome is seen at one pole as a deeply staining sphere, in the astral area of which the spermatozoön lies. The section does not pass through the centrosome of the other pole. The chromosomes quickly move to the equatorial region and the spindle remains in this condition until the egg is laid. Fig. 5 is a polar view of such a spindle and shows the number of chromosomes to be ten, a number confirmed by many counts. Fig. 6 represents the characteristic appearance of a section of a uterine egg showing the entire spindle with the chromosomes in the equatorial plate. The spermatozoön is present in the next section of this egg.

Such in brief is the history of the formation of the uterine spindle. It is an enormous structure occupying the entire central region of the egg; its astral radiations stretching out from either pole almost to the egg membrane. The centrosomes appear as deeply staining spheres, in iron-hæmatoxylin preparations, but in safranin-Lichtgrün each sphere is resolved into a number of hollow vesicles staining with the acid dye.

At this time the spermatozoon may be seen in almost any part of the egg, between the spindle and the periphery. It may lie in the astral area or very close alongside of the spindle and near the chromosomes. The spermatozoön shows no change in structure from the sickle-shaped form in which it first appears in the impregnated ovum.

This is the type of spindle formed in the vast majority of uterine eggs, and in animals secured under the best conditions, only this type is found. However, in some adult worms, in addition to this so-called normal spindle, there occur other spindles which have the same general structural features, but show anomalies of various sorts. Thus the axis of the spindle may be bent (Fig. 7) or even broken at the equator and the chromosomes may be scattered irregularly on or near the spindles. Tri- and tetra-polar spindles of a variety of forms are also found, two of which are shown in Figs. 8 and 9. None of these spindles

so far as we have observed completes the division cycle; at least not while the ovum is still in the uterus.

We believe that these abnormal spindles are the result of unfavorable conditions arising principally from not removing the worms from the whelk soon enough after the latter are taken from the sea, or perhaps from some other pathogenic cause, for they do not have any part in the normal development of the egg. Whether or not such eggs develop after being laid is a question we have not entered into. It may be that the presence of a few such spindles among normal ones in the uterus has led other observers to believe that they were stages in the supposed disintegration of the normal uterine spindle. In fact, we were inclined toward such an interpretation until, largely as a result of exercising greater care in handling the living material, we obtained worm after worm in which the uterus does not show a single abnormal mitotic figure.

III. The Laid Egg.

The condition of the laid egg of *Planocera* has been described both by Wheeler ('94) and by Surface ('07). According to Wheeler, the nucleus returns to a resting condition during or just before the egg is laid, and Surface states that it contains a large germinal vesicle which is situated slightly to one side of the center. We are unable to confirm these observations. In the first place, we find a considerable variation in the condition of the freshly laid eggs. Usually such eggs show that the so-called uterine spindle has undergone, or is in the process of undergoing, contraction, just prior to its migration to the surface to give rise to the first polar body.

The varying conditions of which we have just spoken consist almost altogether in the state of contraction or shortening shown by the spindle at the time the egg is laid. The most extreme cases are those in which the shortening is completed and the spindle has already migrated to the surface of the egg. Indeed, we have one lot of eggs (laid July 25, 1912) which were killed immediately after they were laid, and in which one occasionally finds eggs having the first polar body well started or completely formed. These variations in the condition of the spindle

are easily explained on the basis of the assumption that there is considerable variation in the time of oviposition on the part of the different individuals; and this in turn is undoubtedly influenced by the conditions under which the animal is kept just prior to the laying of the eggs.

We have been somewhat at a loss to account for the observations of Wheeler and Surface, but believe that they may be explained in any one of several different ways. If a lot of freshly laid eggs, in which the contraction of the spindle has progressed to an advanced stage, be examined under the low or medium powers of the microscope, many of the eggs will appear to possess germinal vesicles. However, it can be shown conclusively that under such conditions the small contracted spindle is practically invisible, and that what one really observes in these living eggs is the relatively clear area of protoplasm in which the small spindle lies. This can be shown beautifully by staining the fresh eggs with neutral red, and examining them under the 4 mm. objective. Under such conditions, the contracted spindle stands out with great brilliancy, and one can easily follow the course of its migration to the surface of the egg and observe the formation of the first polar body.

That eggs possessing germinal vesicles may be laid we do not deny, for occasionally they are; but we can affirm that such vesicles are never formed in an egg after it has produced the uterine spindle. They are merely the non-transformed germinal vesicles of ovarian eggs, and their presence at this stage is to be explained by the fact that eggs possessing them either have recently been penetrated by the spermatozoön, or have not been inseminated at all. There is no room for doubt on this point. These germinal vesicles are in every particular similar to those of the ovarian egg—so much so that we have deemed it unnecessary to draw one for illustration, but refer the reader to Fig. 1. Furthermore, we have found at least two freshly laid eggs which contained germinal vesicles undergoing transformation to form the first maturation spindle. Each of these eggs showed a condition that indicated recent insemination, for the spermatozoön was lying close to the egg membrane and had not yet undergone the transformation necessary to produce the vesicular

condition, so characteristic of the sperms in the ordinary eggs, which already possess the completed spindle. It should also be noted that we have occasionally observed non-fertilized uterine eggs which contained the unmodified germinal vesicle.

What we have just stated is further confirmed by observations on eggs that were fixed soon after they were laid. In certain phases of the contraction stage the spindle fails almost entirely to take up the hæmatoxylin stain, so that in studying such material one gains the impression that in at least some of the eggs the spindle has retrograded, or has even completely disappeared. However, if sections from the same series are stained with safranin and Lichtgrün the spindle stands out clearly and distinctly and is found in practically every egg.

This, together with the further fact that abnormal spindles are sometimes found, might easily lead the observer to conclude that a degeneration of the uterine spindle takes place. However, a careful study of a complete series of stages will convince anyone that such is not the case. We have been able, by the means of such a series, to follow the entire history of the uterine spindle, from the time of its first appearance up to the formation of the first polar body. The early phases of the spindle have already been sufficiently dealt with, and the rest of the history, from the contraction of the spindle to the formation of the polar bodies, follows.

The contraction of the spindle is a characteristic phase of the process of maturation, and occurs at about the time of oviposition or shortly thereafter. The spindle shortens to less than half its original length (cf. Figs. 6 and 10). During the shortening the astral centers of the spindle draw closer together and gradually stain less and less deeply. This is probably what Selenka and others refer to when they speak of the polar suns drawing together and growing faint. The end result of the shortening is the production of a short, relatively thick spindle. The shortening usually begins when the spindle starts to move to the surface, but in some eggs the process is completed while the spindle holds an approximately central position (Fig. 10).

This process of contraction is by no means unique for *Planocera*, but is characteristic of the first maturation spindle of several

other forms, both among vertebrates and invertebrates. Conklin ('02 and '12) has described this same phenomenon in *Crepidula*, and in *C. plana* he states ('12) that the first maturation spindle undergoes a reduction from about 42 μ to 24 μ in length. He ('02) cites the following references in which the shortening process is known to occur: *Ascaris* (Boveri, '87), *Branchipus* (Bauer, '92), *Ophryotrocha* (Korschelt, '95), *Myzostomum* (Wheeler, '95), *Cerebratulus* (Coe, '99), *Polychœrus* (Gardiner, '98), *Axolotl* and *Triton* (Carnoy and Lebrun, '99, as seen in their figures 110 and 112). To this list we can now add *Planocera*, and also *Graffilla* (Patterson, '12).

Conklin's suggestion concerning the cause of this phenomenon is of interest here. He believes that it is due primarily to the peripheral movement of the spindle, and that its chief result is the formation of a much smaller polar body than would be produced if the spindle retained its original length.

During the progress of the contraction, the spindle moves toward the periphery of the egg, and upon reaching the surface its distal end comes in contact with the egg membrane, which apparently moves down to meet it. In the meantime the chromosomes undergo division, and the two groups have reached a late anaphase (Fig. 12). A protrusion on the surface of the egg then appears and into this elevation the distal end of the spindle is pushed (Fig. 13). Subsequently a typical polar body is cut off. We have found one unusually clear case of the first polar body, in which the ten chromosomes are easily seen (Fig. 14). Within another hour the second polar body is thrown off in the usual manner, and fertilization and cleavage then follow. In other words, the whole process of maturation and fertilization in this animal is quite typical of that of many other forms.

In conclusion we may state briefly the results of our study. We find that the so-called "uterine" or "aborting spindle" of *Planocera* is initiated by the process of insemination; that it is nothing more nor less than the first phases of a rather typical maturation spindle, and that consequently it undergoes a shortening while moving to the surface of the egg to give rise to the first polar body. It may be going too far to suggest that probably the uterine spindles which have been described in

several other forms are of this same nature; nevertheless, we are inclined to believe that a careful study of the spindle in such organisms will show it to be only the first maturation spindle.

WOODS HOLE, MASS.,
August 3, 1912.

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Description of Plates.

Plate I.

The figures are camera drawings made at table level. Figs. 1–5 inclusive were made with 1.5 mm. Zeiss apochromatic objective and No. 12 compensating eye-piece; the remaining figures were made with the same objective and No. 6 eye-piece.

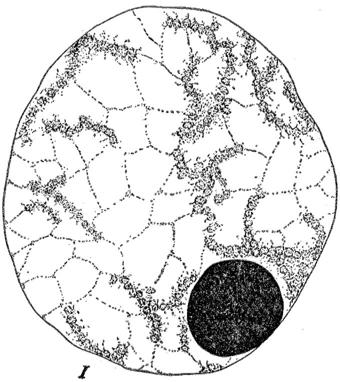
Fig. 1. Resting nucleus with large nucleolus characteristic of growth period.

Fig. 2. Early stage in prophase of uterine spindle. *C*, chromosomes.

Fig. 3. Prophase showing chromosomes fully formed about the fading nucleolus.

Fig. 4. Spindle showing typical tetrad chromosomes. *S*, spermatozoön.

Fig. 5. Polar view of equatorial plate of uterine spindle showing ten chromosomes.



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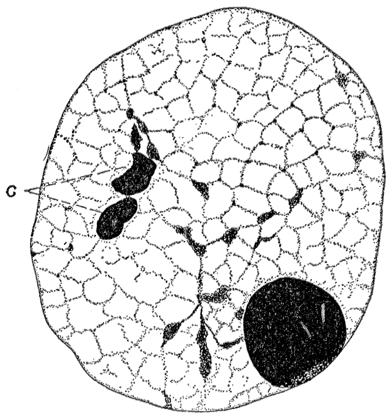

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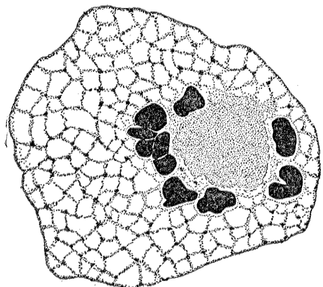

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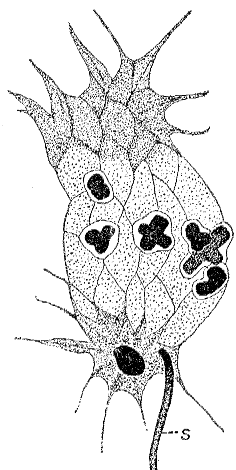

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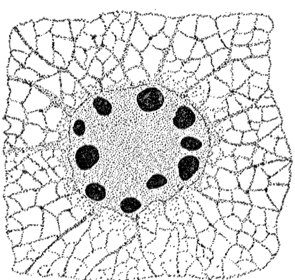

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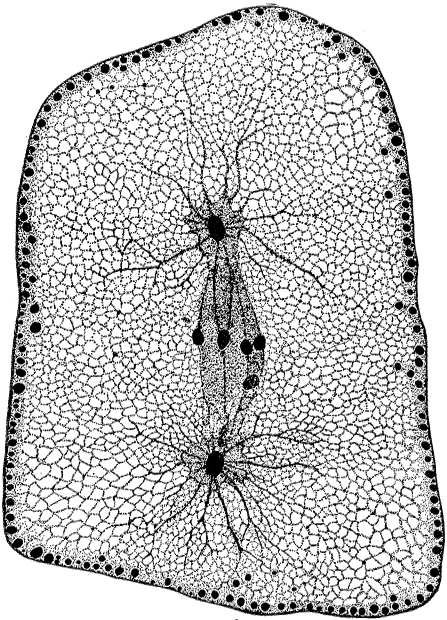
PLATE II.

FIG. 6. Section of entire uterine egg showing spindle.

FIGS. 7, 8 and 9. Abnormal spindles. *S*, spermatozoön.



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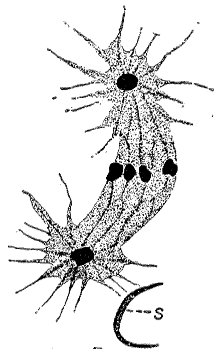

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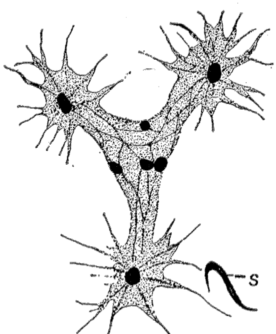

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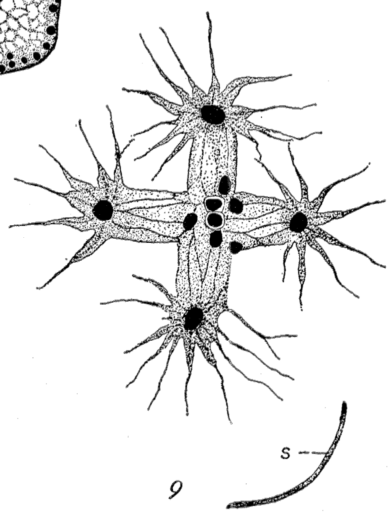

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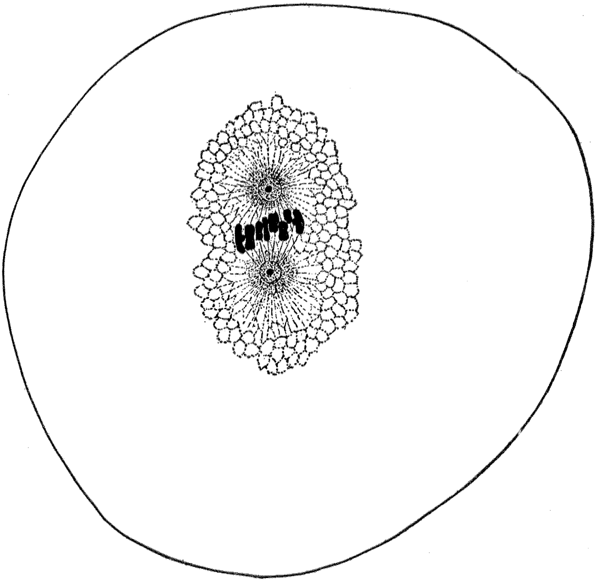
PLATE III.

FIG. 10. Laid egg showing spindle in contracted condition.

FIG. 11. Stages in migration of spindle to periphery of egg.



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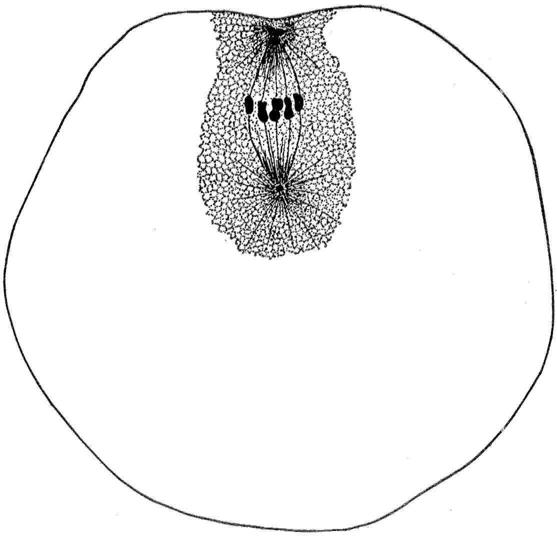

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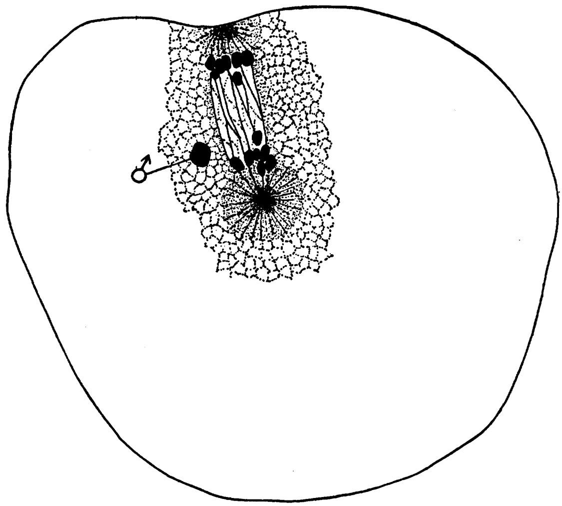
PLATE IV.

FIG. 12. Stages in migration of spindle to periphery of egg.

FIG. 13. Extrusion of first polar body.



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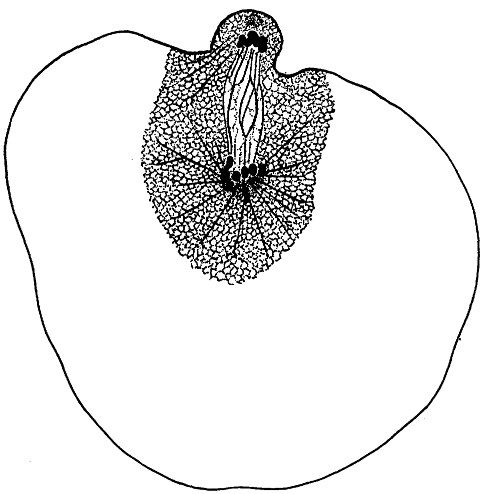

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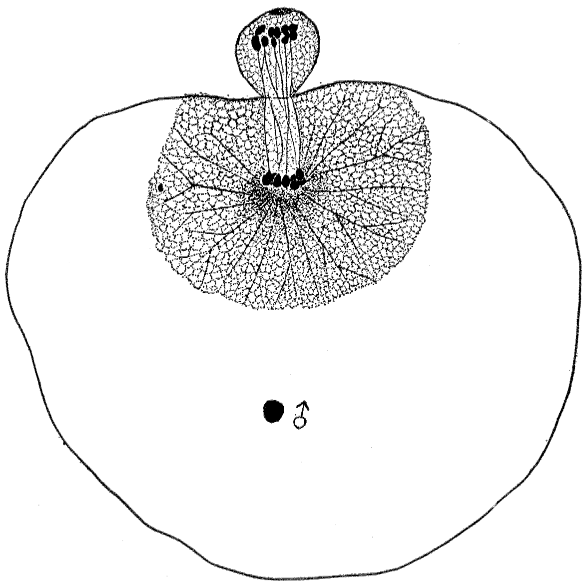
PLATE V.

FIG. 14. Extrusion of first polar body.



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